

Original Article

IMPAIRED PULMONARY $\dot{V}O_2$ KINETICS IN CYSTIC FIBROSIS DEPEND ON EXERCISE INTENSITY

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Running Title: $\dot{V}O_2$ kinetics in young people with CF

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ABSTRACT

Purpose: To investigate the effects of mild-to-moderate cystic fibrosis (CF) on the pulmonary oxygen uptake ($\dot{V}O_2$) kinetics of 7 pediatric patients (13.5 ± 2.8 y) versus 7 healthy matched controls (CON; 13.6 ± 2.4 y). We hypothesized that CF would slow the $\dot{V}O_2$ kinetic response at the onset of moderate (MOD) and very heavy (VH) intensity cycling. **Methods:** Changes in breath-by-breath $\dot{V}O_2$, near-infrared spectroscopy-derived muscle deoxygenation ([HHb]) at the *m. vastus lateralis* and thoracic bioelectrical impedance-derived heart rate, stroke volume index (SVI) and cardiac index (CI) were measured during repeat transitions to MOD (90% of the gas exchange threshold) and VH ($\Delta 60\%$) intensity cycling exercise. **Results:** During MOD, the phase II $\dot{V}O_2 \tau$ ($p=0.84$; effect size (ES) = 0.11) and overall mean response time (MRT) ($p=0.52$; $ES=0.11$) were not significantly slower in CF versus CON. However, during VH exercise, the phase II $\dot{V}O_2 \tau$ ($p=0.02$, $ES=1.28$) and MRT ($p=0.01$, $ES=1.40$) were significantly slower in CF. Cardiac function, central O_2 delivery (SVI and CI) and muscle [HHb] kinetics were unaltered in CF. However, the arterial-venous O_2 content difference ($C_{(a-\bar{v})O_2}$) was reduced during VH at 30 s ($p=0.03$, $ES=0.37$), with a trend for reduced levels at 0 s ($p=0.07$, $ES=0.25$), 60 s ($p=0.05$, $ES=0.28$) and 120 s ($p=0.07$, $ES=0.25$) in CF. Furthermore, $\Delta C_{(a-\bar{v})O_2}$ significantly correlated with the VH phase II $\dot{V}O_2 \tau$ ($r = -0.85$; $p=0.02$) and MRT ($r = -0.79$; $p=0.03$) in CF only. **Conclusion:** Impairments in muscle oxidative metabolism during constant work rate exercise are intensity-dependent in young people with mild-to-moderate CF. Specifically, $\dot{V}O_2$ kinetics are slowed during VH but not MOD cycling and appear to be mechanistically linked to impaired muscle O_2 extraction and utilization.

Keywords: oxidative muscle metabolism; pulmonary disease; near-infrared spectroscopy; oxygen delivery; pediatrics.

INTRODUCTION

Maximal $\dot{V}O_2$ uptake ($\dot{V}O_{2\max}$) is clinically important in patients with cystic fibrosis (CF), given associations with prognosis (26), risk of hospitalization (25) and health-related quality of life (11). $\dot{V}O_{2\max}$ by definition does not, however, represent the rate at which aerobic energy transfer adapts to the changing metabolic demands facing O_2 transport and utilization during everyday life. In contrast, assessing the dynamic adjustment in pulmonary $\dot{V}O_2$ [time constant (τ) for the primary component (phase II)] at the onset of exercise provides a non-invasive insight into muscle O_2 consumption dynamics (21) and the breakdown of muscle phosphocreatine (PCr) (30, 4). Consequently, this parameter can provide insight into the factor(s) mediating muscle metabolic function and the integration of the respiratory, cardiovascular and muscular systems at the onset of exercise. Compared to healthy children (for a review see (2)), there is limited evidence in young people with CF for the $\dot{V}O_2$ kinetic response and its regulating mechanism(s)

Slower $\dot{V}O_2$ kinetics have been reported in people with CF during incremental (34, 17), pseudo-random binary sequence (PRBS) (22) and constant work rate (CWR) exercise (19, 1). However, a similar $\dot{V}O_2$ kinetic response to healthy controls (11-15 y) has been documented during intense exercise (7). Methodological issues may explain these disparities. Firstly, during incremental and PRBS exercise the phase II portion of the $\dot{V}O_2$ response was not isolated, which is critical to reflect the kinetics of muscle O_2 consumption (21). Secondly, the CWR exercise study by Hebestreit and colleagues (19) did not prescribe work rate within physiologically defined exercise intensity domains. Furthermore, semi-recumbent cycling was used which may negate muscle O_2 delivery during exercise, and a mixed age group of 10-33 y, which would comprise a range of pulmonary function characteristics, were tested (19).

According to the Fick principle, the rate of adjustment in $\dot{V}O_2$ is dictated by O_2 delivery and utilization mechanisms but few studies have applied this to understand how CF modifies the $\dot{V}O_2$ kinetic response to CWR exercise (42). Slower $\dot{V}O_2$ kinetics in CF have been linked to impaired O_2 delivery (19), inferred from arterial O_2 saturation ($SpO_{2\%}$). Although children with CF may present with early signs of cardiovascular abnormalities (18, 38, 29), impaired skeletal muscle oxidative capacity in CF is reported (30, 34, 40, 15, 12). The near-infrared spectroscopy (NIRS)-derived muscle deoxygenation ($\Delta[HHb]$) signal provides insight into the ratio of local muscle O_2 delivery to muscle O_2 utilization. Thus, changes in muscle HHb are considered to represent changes in muscle O_2 extraction dynamics during exercise (e.g. 16). Although it has been hypothesised that more rapid muscle HHb dynamics would be evident in the face of reduced central or muscle O_2 delivery (16), children and adolescents with CF do not appear able to compensate in this manner during incremental exercise (30, 34). This raises questions regarding the capacity of CF skeletal muscle to increase muscle O_2 extraction during exercise, but this has yet to be evaluated alongside $\dot{V}O_2$ kinetics during CWR cycling exercise.

The aim of the study was to characterize the pulmonary $\dot{V}O_2$ kinetic response of children and adolescents with mild-to-moderate CF at the onset of moderate (MOD) and very heavy (VH) intensity cycling exercise. It was hypothesized that: 1) a longer phase II $\dot{V}O_2$ τ at the onset of MOD and VH exercise would be evident in CF; 2) slower cardiac output (\dot{Q}) and more rapid $[HHb]$ kinetics would be evident in CF during MOD and VH exercise; and 3) slower $\dot{V}O_2$ kinetics would relate to reduced \dot{Q} and altered muscle $\Delta[HHb]$ dynamics in the CF group.

MATERIALS AND METHODS

Study Participants. Seven young Caucasian individuals with stable, mild-to-moderate CF (Tables 1 and 2) and 7 controls (CON) (Table 2) participated. Inclusion criteria comprised a diagnosis of CF based on clinical features, an abnormal sweat test (sweat chloride > 60 mmolL⁻¹ / 100 mg sweat) and genotyping. Stable pulmonary function within 10% of best in the preceding 6 months and no symptomatic increase or weight loss within 2 weeks was also mandatory. Unstable non-pulmonary comorbidities and/or acute infection warranted exclusion. Ethics approval was granted by the South West NHS Research Ethics Committee. Informed written consent and assent was obtained from parent(s)/guardian(s) and participants.

Table 1. Baseline clinical characteristics for the young CF patients upon initiation into the study.

Variable	Value (mean \pm SD)	Range
<u>CFTR genotype:</u>		
Homozygote Δ F508	4	
Δ F508/ 2184delA	1	
Δ F508/ G551D	1	
Δ F508/ P67L	1	
<i>Chronic P. Aeruginosa</i> infection ^a	“chronic,” $n = 1$; “intermittent,” $n = 2$	“free,” $n = 3$ “never,” $n = 1$
Shwachman score	85 ± 5	80-90
Northern score ^b	4 ± 1	3-6
Pancreatic insufficient	$n = 7$	
CF-related diabetes	$n = 1$	
CF-related liver disease	$n = 1$	
IVABs (days in last year)	11 ± 9	0-24

Values are means \pm SD, with the range also displayed where suitable, unless otherwise stated.

CFTR, cystic fibrosis transmembrane conductance regulator; *P. Aeruginosa*; *Pseudomonas Aeruginosa*; Shwachman score - scoring 4 separate aspects of the disease profile; general activity; physical examination; nutritional status; and chest radiographic findings, using the most recent clinical review information. A total of 100 points represents a perfect score of health; IVABs, intravenous antibiotics; ^aAccording to Leeds Criteria, “chronic”, > 50% of the preceding 12 months were *P. aeruginosa* culture positive; “intermittent”, $\leq 50\%$ of the preceding 12 months were *P. aeruginosa* culture positive; “never”, no growth of *P. aeruginosa* for the previous 12 months, having previously been *P. aeruginosa* culture positive; “free”, *P. aeruginosa* has never been cultured.

^b Provides evidence of radiographic chest findings. Maximum score is 20, with 20 being the most severe.

Table 2. Baseline anthropometric, pulmonary function and maximal cardiopulmonary exercise testing data for young patients and healthy age- and gender-matched control participants upon initiation into the study.

Variable	CF Mean \pm SD	CON Mean \pm SD	<i>p</i> -value	ES (<i>d</i>)
Gender	5 M, 2 F	5 M, 2 F	-	-
Age (y)	13.5 \pm 2.80	13.6 \pm 2.40	0.93	-0.04
Stature (m)	1.61 \pm 0.20	1.62 \pm 0.17	0.92	-0.05
Body mass (kg)	60.7 \pm 22.8	52.4 \pm 17.8	0.46	0.38
BMI (kg·m ²)	22.6 \pm 4.5	19.4 \pm 2.9	0.14	0.79
FFM (kg)	49.5 \pm 19.9	40.1 \pm 12.5	0.30	0.54
FVC (L)	3.91 \pm 1.29	4.08 \pm 1.50	0.82	-0.12
FVC (% predicted ^a)	106 \pm 10	107 \pm 17	0.82	-0.11
FEV ₁ (L)	3.27 \pm 1.00	3.59 \pm 1.26	0.61	-0.26
FEV ₁ (% predicted ^a)	102 \pm 6	110 \pm 12	0.17	-0.72
	-	-	-	-
<i>CPET parameters</i>				
$\dot{V}O_{2\max}$ (L·min ⁻¹)	2.08 \pm 0.74	2.51 \pm 0.91	0.34	-0.49
$\dot{V}O_{2\max}$ (mL·kg ⁻¹ ·min ⁻¹)	34.30 \pm 8.88	47.75 \pm 3.56	< 0.01*	-1.79
$\dot{V}O_{2\max}$ /FFM (mL·kg ⁻¹ ·min ⁻¹)	51.87 \pm 34.90	65.52 \pm 24.65	0.42	-0.42
$\dot{V}O_2$ at the GET (L·min ⁻¹)	1.09 \pm 0.31	1.38 \pm 0.48	0.20	-0.67
GET _% (% of $\dot{V}O_{2\max}$)	53.7 \pm 6.4	55.2 \pm 3.3	0.57	-0.28
Ramp PPO (W)	162 \pm 61	208 \pm 86	0.27	-0.58
Ramp TTE (s)	546 \pm 111	729 \pm 113	0.01*	-1.52
SpO _{2%} (%)	95 \pm 3	98 \pm 1	0.04*	-1.23

Values are means \pm SD unless otherwise stated. M, males; F, females; BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; CPET, cardiopulmonary exercise testing; $\dot{V}O_{2\max}$, maximal oxygen uptake; FFM, fat-free mass; GET, gas exchange threshold; MRT, mean response time; PPO, peak power output; TTE, time to exhaustion; S_{max}, supramaximal verification phase; SpO_{2%}, arterial oxygen saturation. ^aAccording to Stanojevic *et al.* (2009).

Experimental protocol

Participants attended the laboratory five times over a two week period, at a similar time of day and separated by 24-48 h. Participants were advised to arrive rested and hydrated, > 2 h postprandial and having refrained from caffeine (> 2 h). All exercise was performed on a cycle ergometer (Lode, Groningen, The Netherlands or Lode Corival (Pediatric), Groningen).

Visit 1: CPET protocol. Following anthropometric and pulmonary function measurements, a combined ramp incremental and supramaximal (S_{max}) CPET was used to determine $\dot{V}O_{2\max}$ and the gas exchange threshold (GET) (32, 33). This protocol involved an exhaustive ramp incremental (10-

25 W·min⁻¹) cycling test with a subsequent S_{\max} (110% peak power output (PPO)) test to exhaustion, to confirm a valid $\dot{V}O_{2\max}$ measurement. Following a 3 min warm-up (20 W), participants completed the incremental test to the point of volitional exhaustion, maintaining a cadence of 70-80 rpm throughout. Exhaustion was defined as a ≥ 10 rpm drop in cadence for 5 consecutive seconds, despite strong verbal encouragement. Active (5 min cycling at 20 W) and then passive seated recovery (10 min) then preceded the S_{\max} test. S_{\max} verification consisted of a 3 min warm-up (20 W), followed by a 'step' transition to a CWR corresponding to 110% PPO. Upon volitional exhaustion (defined above), a 5 min active recovery (slow cycling at 20 W) concluded the combined CPET session.

Visits 2-5: CWR exercise. For each visit the participants completed MOD and VH CWR exercise tests, comprising 6 min unloaded pedalling (10 W), followed by transitions to elicit $\dot{V}O_2$ amplitudes corresponding to 90% GET and $\Delta 60\%$ (60% of the difference between the GET and $\dot{V}O_{2\max}$) for 6 min. This equated to MOD work rates of 58 ± 24 W and 73 ± 35 W for CF and CON, respectively. During VH, CF and CON cycled at 121 ± 43 W and 150 ± 64 W, respectively. Thirty minutes rest separated the MOD and VH transitions. Each set of MOD and VH transitions was performed on separate days, separated by ≥ 48 h and within a 2 week period.

Experimental measures

Anthropometry and pulmonary function. Body mass (Seca 220; Vogel & Halke, Hamburg, Germany) and stature (Seca 220; Vogel & Halke, Hamburg, Germany) were measured to the nearest 0.01 kg and 0.01 m. Skinfold measurements (Harpender; British Indicators, Burgess Hill, UK) were used to estimate percentage body fat (37). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁) were assessed using spirometry (Micromedical Microloop 3535, Numed, Sheffield, UK), and expressed as a percentage predicted using appropriate reference values (38).

Gas exchange and pulse oximetry. Breath-by-breath changes in gas exchange and ventilation were determined using a metabolic cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany), which was calibrated each test using gases of known concentration and a 3 L syringe (Hans Rudolph, Kansas City, MO). Fingertip $\text{SpO}_2\%$ was measured on a beat-by-beat basis at the fingertip using pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc., USA).

Near-infrared spectroscopy. A near-infrared spectrometer (Portamon, Artinis Medical Systems) was used to non-invasively measure [HHb] at the *m. vastus lateralis*. Details regarding this system have been outlined in our previous work in young people with CF during ramp incremental exercise (34). Briefly, this system consists of an emission probe, with three light sources emitting two wavelengths of light (760 and 850 nm) and a photon detector. Following cleaning and shaving of the area of interrogation, the wireless emitter-detector unit was placed over the muscle belly, midway between the greater trochanter and lateral epicondyle of the femur. After marking of the placement area, the device was secured with tape (Kinesio[®] Tex) and a dark elastic bandage to minimize extraneous light interference with the near-infrared signal. The intensity of incident and transmitted light was recorded at continuously at 10 Hz.

Thoracic Impedance. Beat-by-beat changes in heart rate (HR), SV and \dot{Q} were measured using a bioelectrical impedance cardiography system (PhysioFlow, PF-05, Manatec Biomedical, Paris, France) that has previously been used in CF (e.g. 2). This technique uses a high-frequency (75 kHz) and low-magnitude (1.8 mA) current across the thorax, to enable changes in thoracic impedance during the cardiac cycle to be recorded. Following preparation of the skin sites, electrodes were positioned on the forehead, base of the neck and above the supraclavicular fossa, and two positioned on the xiphoid process. Another set of two electrodes were used to determine a single electrocardiograph signal at the V1 and V6 positions.

CPET parameters of aerobic function. The highest 15 s averaged $\dot{V}O_2$ from the ramp and S_{\max} tests represented $\dot{V}O_{2\max}$ (32) and was normalized to fat-free mass (FFM) using the ratio standard method. The GET was identified using the V-slope method (5) and confirmed through visual inspection of the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$.

Pulmonary $\dot{V}O_2$ kinetics. Breath-by-breath changes in $\dot{V}O_2$ were analysed using methodology previously described by our laboratory (3, 8). Briefly, the four repeat transitions for both MOD and VH were linearly interpolated to 1 s, time aligned to exercise onset (i.e., $t = 0$ s) and ensemble averaged. The 1 s averaged $\dot{V}O_2$ response for the MOD and VH conditions for each participant were then baseline corrected, by subtracting the mean $\dot{V}O_2$ between -60 and -5 s from the exercise response. The duration of phase I was visually assessed to account for the cardio-dynamic contribution to the $\dot{V}O_2$ kinetic response. The first 21 ± 3 s and 17 ± 4 s of the MOD data and the first 19 ± 5 s and 16 ± 2 s for VH were omitted in CF and CON participants, respectively. The phase II portion of the $\dot{V}O_2$ response was then characterised using Equation 1 (GraphPad Prism; GraphPad Software, San Diego, CA):

$$\dot{V}O_{2(t)} = \Delta \dot{V}O_{2A} \cdot (1 - e^{-(t-TD)/\tau}) \quad \text{Equation 1.}$$

where $\dot{V}O_{2(t)}$, $\Delta \dot{V}O_{2A}$, TD , and τ represent the value of $\dot{V}O_2$ at a given time (t), the amplitude change in $\dot{V}O_2$ from baseline to its asymptote, time delay, and the time constant of the response, respectively.

The MRT was derived to describe the overall kinetics during both MOD and VH, by constraining the TD in Equation 1 to the onset of phase I and fitting to end-exercise. The functional $\dot{V}O_2$ gain of phase II was determined by dividing the phase II $\dot{V}O_2$ amplitude by the change in work rate above baseline. End-exercise $\dot{V}O_2$ gain was calculated in a similar manner. For VH exercise, the $\dot{V}O_2$

slow-component onset and amplitude were determined in line with our previous work (3, 8). Briefly, Equation 1 was first fit up to the initial 60 s of exercise and then increased iteratively by 1 s to the end of the exercise bout (LabView, v 6.1, National Instruments, Newbury, UK). The best fit curve for the phase II portion of the response was established using: 1) a plot of the $\dot{V}O_2$ τ against time, to identify the point at which the influence of the $\dot{V}O_2$ slow component lengthened the estimated τ following an initial plateau; and 2) deviation from an optimal fitting of the model as judged by a systematic departure of the model's residuals. The phase II parameter estimates from equation 1 were then resolved by least-squares non-linear regression (GraphPad Prism, GraphPad Software, San Diego, CA). The magnitude of the $\dot{V}O_2$ slow component was calculated as the difference between the mean of the final 30 s at 6 min of exercise and the phase II asymptote and was expressed in both absolute terms and relative to end-exercise $\dot{V}O_2$.

Muscle oxygenation kinetics. NIRS data were collected at 10 Hz, interpolated to 1 s intervals and expressed as a change, in arbitrary units (a.u.), from baseline. Subsequently, [HHb] profiles were 5 s averaged, time aligned to exercise onset and ensemble averaged to yield a single response. The dynamics of the primary and slow-component phases of the [HHb] response were modelled in a similar manner to $\dot{V}O_2$, with slight modification. The exponential-like increase in [HHb] after the onset of exercise occurred after a discernible delay. The time at which the exponential-like increase in [HHb] commenced was identified as the point of a 1 SD increase above baseline (3). Equation 1 was then applied to resolve the [HHb] TD and τ following removal of the data preceding the exponential-like increase. The [HHb] MRT was calculated by summing TD and τ to provide an overall description of the kinetics in the primary phase.

Heart rate, stroke volume, cardiac output and $C_{(a-\bar{v})}O_2$. Beat-by-beat changes in HR, SV and \dot{Q} were linearly interpolated to 1 s, time aligned and ensemble averaged to 30 s. The arterial-venous O_2 content difference [$C_{(a-\bar{v})}O_2$] was estimated via rearrangement of the Fick equation [$C_{(a-\bar{v})}O_2 =$

$\dot{V}O_2 / \dot{Q}$]. SV and \dot{Q} were normalized to FFM (12) using the ratio standard method, to determine the cardiac index (CI) and SV index (SVI). The $\dot{Q} / \dot{V}O_2$ ratio was used to provide an index of muscle O_2 availability relative to metabolic rate.

Statistical analyses

Independent samples *t*-tests examined mean differences between CF and CON. Additionally, effect size [*ES* (*d*)] statistics determined the magnitude of the effect, using a pooled SD. The magnitude of the difference between variables of interest were explored using *ES* thresholds of trivial (< 0.2), small (> 0.2), moderate (> 0.5), large (> 0.8), and very large (> 1.0) (9). Changes in HR, SV, \dot{Q} and $C_{(a-\bar{v})}O_2$ were analysed using mixed model ANOVA. Significant interactions were followed up using independent samples *t*-tests. Pearson's correlation coefficients assessed relationships between $\dot{V}O_2$ kinetics and mechanistic parameters of O_2 delivery and utilization. Statistical analyses were performed using SPSS (version 19.0, SPSS, Chicago, IL), with the null-hypothesis rejected at alpha level of 0.05.

RESULTS

Maximal cardiopulmonary exercise testing

Descriptive characteristics and CPET data are presented in Table 2. There were no differences in body size and composition and lung function between CF and CON. $\dot{V}O_{2\max}$ was reduced in CF compared with CON when normalized using body mass but not FFM.

Pulmonary $\dot{V}O_2$ kinetics

The $\dot{V}O_2$ responses during MOD and VH are presented in Figure 1 and the kinetic parameters in Table 3. There was no significant difference in baseline $\dot{V}O_2$ between the groups for either MOD or VH exercise (Table 3). For MOD, CF had no influence on either the phase II τ , TD or MRT. However, the phase II $\dot{V}O_2$ gain was lower in CF. During VH exercise, the $\dot{V}O_2$ MRT and phase II

τ were slower in CF. The phase II *TD*, amplitude and gain and end-exercise $\dot{V}O_2$ were not altered in CF. A $\dot{V}O_2$ slow-component manifested in all VH responses, however the amplitude was similar between CF and CON.

Table 3. Pulmonary oxygen uptake kinetics in CF and CON during MOD and VH exercise.

Variable	CF Mean \pm SD	CON Mean \pm SD	<i>p</i> -value	ES (<i>d</i>)
<i>Moderate intensity exercise</i>				
Baseline $\dot{V}O_2$ (L·min ⁻¹)	0.62 \pm 0.13	0.68 \pm 0.16	0.47	-0.38
Phase II $\dot{V}O_2$ τ (s)	25.2 \pm 6.7	26.1 \pm 8.4	0.84	-0.11
Phase II $\dot{V}O_2$ <i>TD</i> (s)	21.7 \pm 7.3	14.8 \pm 7.1	0.10	0.90
Phase II $\dot{V}O_2$ amplitude (L·min ⁻¹)	0.42 \pm 0.20	0.64 \pm 0.34	0.16	-0.76
Phase II $\dot{V}O_2$ gain (mL·min ⁻¹ ·W ⁻¹)	8.6 \pm 1.3	10.4 \pm 1.4	0.03*	-1.21
$\dot{V}O_2$ mean response time (s)	44.6 \pm 8.0	41.6 \pm 8.9	0.52	0.33
<i>Very heavy intensity exercise</i>				
Baseline $\dot{V}O_2$ (L·min ⁻¹)	0.68 \pm 0.15	0.76 \pm 0.19	0.37	-0.46
Phase II $\dot{V}O_2$ τ (s)	37.5 \pm 10.8	25.1 \pm 6.5	0.02*	1.28
Phase II $\dot{V}O_2$ <i>TD</i> (s)	14.7 \pm 6.1	14.6 \pm 3.0	0.96	0.03
Phase II $\dot{V}O_2$ amplitude (L·min ⁻¹)	0.97 \pm 0.34	1.26 \pm 0.54	0.25	-0.60
Phase II $\dot{V}O_2$ gain (mL·min ⁻¹ ·W ⁻¹)	8.9 \pm 0.8	9.2 \pm 1.2	0.51	-0.34
$\dot{V}O_2$ slow-component onset (s)	144 \pm 29	117 \pm 21	0.07	0.99
$\dot{V}O_2$ slow-component amplitude (L·min ⁻¹)	0.19 \pm 0.15	0.16 \pm 0.13	0.74	0.17
$\dot{V}O_2$ slow-component relative amplitude (%)	9.0 \pm 6.3	6.7 \pm 3.8	0.43	0.40
End-exercise $\dot{V}O_2$ (L·min ⁻¹)	1.83 \pm 0.59	2.18 \pm 0.83	0.38	-0.46
End-exercise $\dot{V}O_2$ gain (mL·min ⁻¹ ·W ⁻¹)	9.9 \pm 1.3	10.3 \pm 0.9	0.56	-0.30
$\dot{V}O_2$ mean response time (s)	74.6 \pm 19.4	51.6 \pm 8.3	0.01*	1.40

Muscle oxygenation kinetics

The group mean data for [HHb] and the corresponding kinetic parameters are shown in Figure 2 and Table S1 (Supplemental Digital Content 1 – muscle oxygenation kinetics of young CF patients and healthy control participants at the onset of moderate and very heavy intensity cycling exercise), respectively. The [HHb] response of one CF patient (male, 10 y, $\Delta F508$ homozygote) did not display exponential characteristics and was, in addition to their healthy control, excluded from [HHb] analyses. There was no difference between CF and CON for any of the [HHb] kinetic parameters during MOD or VH exercise.

Heart rate, stroke volume index, cardiac index and $C_{(a-\bar{v})}O_2$.

Group mean HR, CI, SVI and $C_{(a-\bar{v})}O_2$ dynamics are presented in Figure 3. No significant time by disease state interaction effect for SVI was evident during either MOD ($p = 0.09$) or VH ($p = 0.27$). During VH there was a significant interaction between time and disease state for HR ($p = < 0.01$), with follow-up comparisons identifying a higher HR in CF at 30 s ($p = 0.04$, $ES = 0.21$). There was a significant main effect for disease state ($p = 0.01$) for CI to be lower in CF during MOD but not VH ($p < 0.05$). There was a time by disease state interaction ($p = 0.03$) for $C_{(a-\bar{v})}O_2$ during VH (Figure 3d), with extraction significantly reduced in CF at 30 s ($p = 0.02$, $ES = 0.37$) and a trend towards reduced values at 0 s ($p = 0.07$, $ES = 0.25$), 60 s ($p = 0.05$, $ES = 0.28$) and 120 s ($p = 0.07$, $ES = 0.25$). $\dot{Q}/\dot{V}O_2$ was not different between the groups for either intensity ($p > 0.05$).

Relationships between $\dot{V}O_2$ kinetics and mechanistic parameters

During MOD, the phase II $\dot{V}O_2$ τ significantly correlated with $\Delta[HHb]$ ($r = 0.84$; $p = 0.04$) in CF, whilst the MOD $\dot{V}O_2$ MRT correlated with ΔSVI in CON ($r = -0.81$; $p = 0.03$). During VH, the $\Delta C_{(a-\bar{v})}O_2$ significantly correlated with the phase II $\dot{V}O_2$ τ ($r = -0.85$; $p = 0.02$) and MRT ($r = -0.79$; $p = 0.03$) in CF. Furthermore, $\Delta\dot{Q}/\Delta\dot{V}O_2$ significantly correlated ($r = 0.78$; $p = 0.04$) with the phase II $\dot{V}O_2$ τ in CF during VH exercise.

DISCUSSION

This is the first study to examine the dynamics of $\dot{V}O_2$ in children and adolescents with mild-to-moderate CF at the onset of MOD and VH intensity cycling exercise, relative to adjustments in central O_2 delivery and localized muscle (*m. vastus lateralis*) O_2 extraction. The novel and original findings from this study are: 1) $\dot{V}O_2$ kinetics were slowed in CF during VH but not MOD; 2) no differences in muscle [HHb] kinetics were found between CF and CON during MOD and VH exercise; 3) during VH exercise only, $C_{(a-\bar{v})}O_2$ was reduced in CF within the initial 60 s of exercise onset, and 4) the change in $C_{(a-\bar{v})}O_2$ during VH exercise was significantly correlated with the phase II $\dot{V}O_2$ τ and MRT in CF. Collectively, these findings support the notion that impaired muscle oxidative metabolism in young CF patients is dependent on the intensity of exercise and principally limited by muscular factors, which limit the extraction and utilisation of O_2 during VH exercise

Contrary to our hypothesis, neither the phase II or overall $\dot{V}O_2$ kinetics were slowed during MOD intensity cycling in young people with CF. This is consistent with early observations in similarly aged patients (11.1-15.3 y) with mild airway obstruction during 6 minutes cycling at $1.7 \text{ W}\cdot\text{kg}^{-1}$ (7). In contrast, two studies have documented slower $\dot{V}O_2$ dynamics during exercise in patients with CF (22, 19), however methodological issues may explain this disparity. Kusenbach *et al.* (22) employed PRBS exercise which fails to isolate phase II of the $\dot{V}O_2$ response. Although Hebestreit *et al.* (19) utilised CWR exercise and isolated phase II, work rate was not prescribed within physiologically defined intensity domains (18). This process meant patients were likely to be exercising across the MOD-severe intensity domains which, if the intensity was above the GET, would be consistent with our present findings of slowed $\dot{V}O_2$ kinetics during VH exercise. Hebestreit and colleagues also used semi-supine exercise (19), which may reduce muscle perfusion and slow $\dot{V}O_2$ dynamics (20). Finally, the combination of adult and paediatric patients (10-33 y) could have contributed to slow

$\dot{V}O_2$ kinetics, since slower phase II kinetics were recently documented in adults with more advanced CF (22 ± 4 y) during submaximal cycling (1).

An interesting finding in this study was that the influence of CF on oxidative muscle metabolism appears to be intensity dependent. This is based on the finding that the phase II $\dot{V}O_2$ τ and MRT were slowed only during VH exercise and the ES was very large (> 1.0). This is of clinical importance, since slower $\dot{V}O_2$ kinetic response will incur a greater O_2 deficit and a greater degree of substrate-level phosphorylation (increased lactic acid and PCr breakdown) and the accumulation of fatigue-inducing metabolites (e.g., inorganic phosphate and hydrogen ions), which may impair exercise tolerance especially during VH exercise in young people with CF. An exercise intensity dependence to the impaired oxidative metabolism in CF corresponds with earlier observations in adolescent patients during a 90 s high-intensity exercise challenge performed within a ^{31}P Magnetic Resonance Spectroscopy scanner environment, but not shorter duration or less intense exercise (39). This may reflect the greater physiological challenge to mitochondrial aerobic metabolism elicited by higher intensities.

The longer phase II $\dot{V}O_2$ τ of patients with CF (10-33 y; FEV₁: 37-98% predicted) has previously been linked to inadequate O_2 delivery, inferred by a significant relationship with SpO₂ (19). In the current study, bulk blood flow, as inferred using the CI (\dot{Q}), was not profoundly altered during either MOD or VH exercise in CF. Furthermore, $\dot{V}O_2$ kinetics were not mechanistically linked to the CI and SVI dynamics in this group of patients, despite previous reports that early signs of cardiac dysfunction may present in paediatric patients with CF (18, 34). Although CFTR is involved in the regulation of cardiomyocyte contraction (36) and gene mutation targeted therapies have been shown to increase SV in adolescents with CF (35), the current findings indicate that central O_2 delivery does not principally limit $\dot{V}O_2$ kinetics in young CF patients. This is further supported by research demonstrating that elevating SpO₂ through the inspiration of hyperoxic gas

does not improve the kinetics of $\dot{V}O_2$ in patients with CF (22). However, it must be acknowledged that only central indices of O_2 delivery, which are relatively poor indicators of O_2 delivery at the local muscle level during exercise (24), were obtained in these studies.

However, considering the findings in the present study, the impaired $\dot{V}O_2$ kinetics during VH exercise were related to the capacity of skeletal muscle to extract and utilize O_2 . For the first time, this study investigated the [HHb] dynamics of young patients with CF during CWR exercise, with similar kinetics observed between the groups. If muscle O_2 availability was limiting oxidative metabolism in CF, a compensatory acceleration in the rate of O_2 extraction would be expected (16). This was not observed in the present study and this finding corresponds with earlier studies during incremental exercise using both NIRS (34) and respiratory mass spectroscopy (30). Whilst this finding shows that the rate of O_2 extraction taking place was not different in CF compared to CON, [HHb] does not reflect the amount of O_2 extraction taking place. This can be physiologically interpreted from the $C_{(a-\bar{v})}O_2$ parameter.

Interestingly, we observed a significant reduction in $C_{(a-\bar{v})}O_2$ in CF during the early stages of VH exercise (see Figure 3D), which corresponds with the timing of the phase II portion of the $\dot{V}O_2$ response, although the corresponding effect sizes were small. Furthermore, $\Delta C_{(a-\bar{v})}O_2$ significantly correlated with the phase II $\dot{V}O_2$ τ and MRT during VH in CF only. These findings suggest that the amount of muscle O_2 extraction and utilization is impaired in this patient group near the onset of exercise and is mechanistically linked to the dynamics of $\dot{V}O_2$. These findings support previous speculations regarding a peripheral limitation slowing $\dot{V}O_2$ kinetics in patients with CF (19). This O_2 extraction and utilization impairment may be explained by structural and functional changes in skeletal muscle that are evident in CF (40, 12, 23). Although a recent study has provided conflicting data (41), slower post-exercise PCr recovery kinetics, measured using ^{31}P phosphorous magnetic resonance spectroscopy, suggest impaired muscle oxidative capacity in both the *m. vastus lateralis*

and forearm muscle (40, 12). More recently, reduced local muscle oxidative capacity was inferred from the recovery of *m. vastus lateralis* O₂ consumption following 15 s of electrical stimulation and subsequent repeated transient arterial occlusions (15). Evidence of CF-specific muscle metabolic abnormalities (lower adenosine triphosphate concentration ([ATP]) and ATP:PCr at rest and significantly higher end-exercise pH values) (40) also support the present suggestions regarding a muscular abnormality in this patient group.

The cause(s) of an intramuscular impairment in CF are currently unknown, although several factors have been proposed. Reduced antioxidant capacity, arising from systemic inflammation and/or oxidative damage, may lower mitochondrial efficiency (39). However, it may also be a consequence of the CFTR genetic mutation. CFTR is expressed in skeletal muscle cells (23) and *in vitro* study of leucocyte mitochondria in patients with CF demonstrates that properties of complex I of the respiratory chain are significantly altered (10). Furthermore, absence of CFTR from skeletal muscle has been shown to dysregulate calcium homeostasis, augment inflammatory or atrophic gene expression signatures and increase diaphragm weakness (14). Conversely, improving CFTR (dys)function using Ivacaftor shows potential to improve aerobic exercise function in adolescents with CF (35). Recent evidence that vascular endothelial (dys)function is associated with a poorer $\dot{V}O_{2\max}$ in young people with CF (28, 29) has been reported. However, the impact of impaired vascular function on the ability of people with CF to deliver O₂ locally for extraction also requires further investigation.

Whilst the present study provides the first robust investigation of the $\dot{V}O_2$ kinetic response in young CF patients, there are a number of limitations to be considered. NIRS exercise measurements in this population have recently been outlined in greater detail elsewhere (34), however include a restricted, heterogenous and superficial area of interrogation and possible inter-site variation in [HHb]. To minimize these limitations, the NIRS device was secured to the same anatomical region

of all participants to eradicate inter-individual regional differences within the *m. vastus lateralis* and [HHb] responses were standardized to the total [HHb] amplitude to provide a physiologic normalisation (6). Furthermore, adipose tissue thickness at the site of interrogation was not measured, which precludes comparing amplitude changes between the groups. Although the utilized thoracic impedance device has been validated in CF patients (27), this technique provides a non-invasive estimate of SV and more detailed echocardiography indices of ventricular function, in addition to further measurements of vascular endothelial function would be insightful. Further, since CFTR is expressed in human vasculature and vascular endothelial dysfunction has been related to $\dot{V}O_{2\max}$ in young CF patients (28, 29), contribution to altered $\dot{V}O_2$ kinetics warrants further investigation. Finally, since muscle fibre type composition and recruitment were not measured herein, discrepancies in fibre type composition and recruitment strategies between the groups cannot be excluded.

These findings help us to further understand how young people with CF respond to the increased metabolic demand during activities of daily living and fatiguing exercise. Whilst children and adolescent with mild-to-moderate CF appear to respond in a similar manner to their healthy counterparts during MOD exercise, the slowed $\dot{V}O_2$ kinetics at the onset of exercise above the GET may well be linked to reduced exercise tolerance, which should be considered by the exercise practitioner when considering exercise prescription strategies for this patient group. Promisingly, identifying the rate limiting determinant(s) of pulmonary $\dot{V}O_2$ kinetics in individuals with CF may provide viable targets for intervention.

To conclude, this study demonstrates that the $\dot{V}O_2$ kinetics of paediatric patients with CF are slowed during VH but not MOD intensity cycling exercise. Impaired skeletal muscle oxidative metabolism in this patient group is intensity dependent and appears to be mechanistically linked to an intrinsic intramuscular impairment which limits O_2 extraction and utilization. Identifying the rate

limiting determinant(s) of pulmonary $\dot{V}O_2$ kinetics in individuals with CF may provide viable targets for intervention in the future.

ACKNOWLEDGMENTS / CONFLICTS OF INTEREST

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Supplemental Digital Content 1.doc Muscle oxygenation kinetics of young CF patients and healthy control participants at the onset of moderate and very heavy intensity cycling exercise.

Figure 1. Mean $\dot{V}O_2$ profile for cystic fibrosis (○ white circles) versus healthy (● black circles) children and adolescents during moderate (A, C) and very heavy (B, D) intensity cycling exercise. Figures C and D provide the normalized to end-exercise so that the differences in the phase II region of the $\dot{V}O_2$ response can be observed. The vertical dotted line illustrates the onset of exercise from a 10 W baseline. Data are presented as 5-s averages

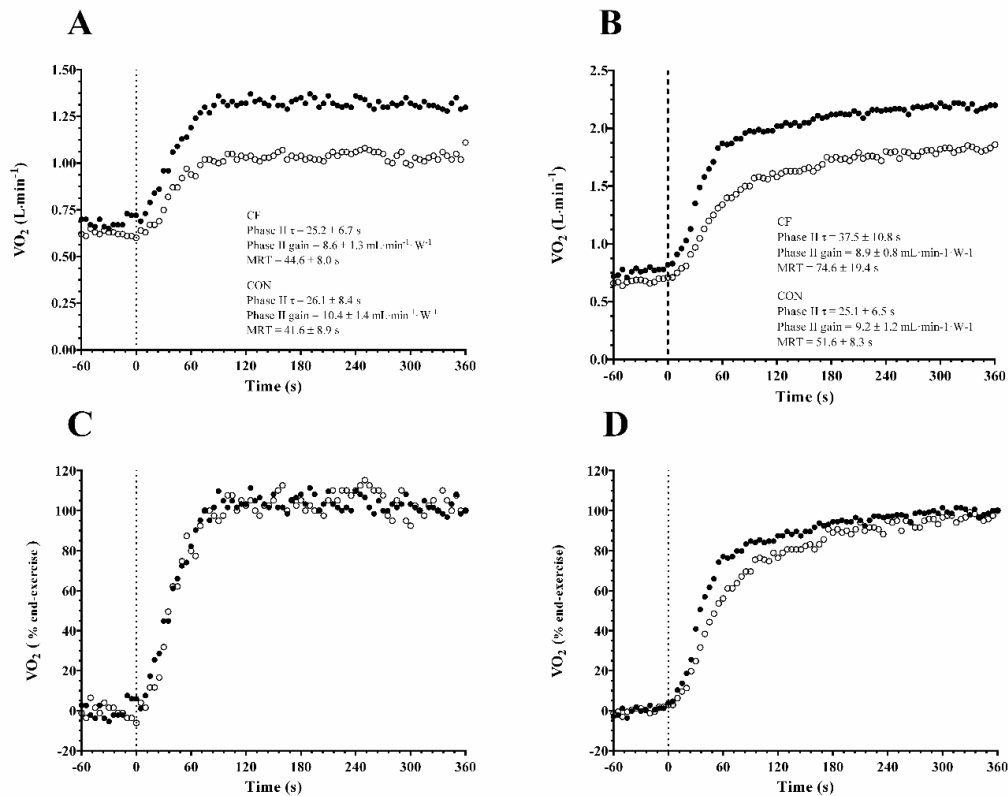


Figure 2. Mean [HHb] profile for cystic fibrosis (○ white circles) and healthy (● black circles) young people during moderate (A,C) and very heavy (B,D) intensity cycling exercise. Figures C and D provide the normalized to end-exercise so that the differences in the phase II region of the [HHb] response can be observed. The vertical dotted line denotes the onset of exercise from a 10 W baseline. Data are presented as 5-s averages.

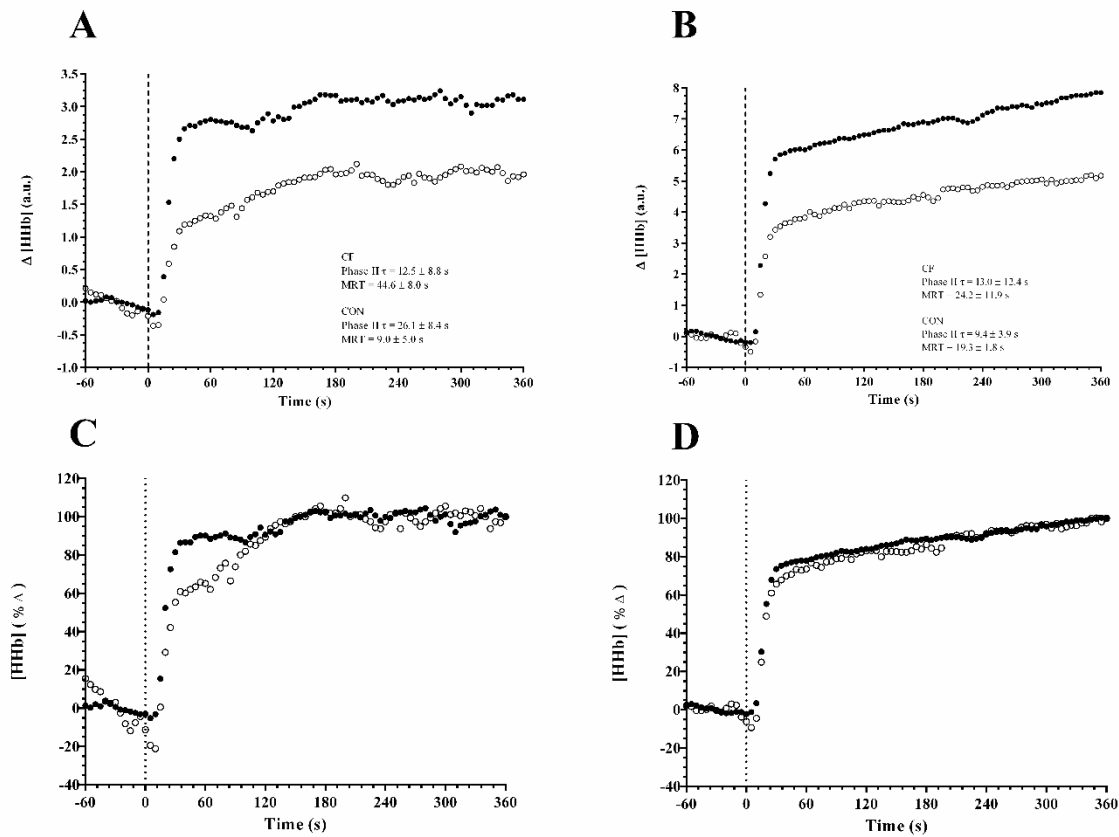


Figure 3. Group mean heart rate (A), fat-free mass (FFM) normalized stroke volume (B), FFM normalized cardiac output (C) and FFM normalized arterial-venous O₂ content difference [$C_{(a-v)}O_2$] (D) dynamics of young cystic fibrosis patients (○ white circles) and healthy age- and gender-matched controls (● black circles) during moderate (1) and very heavy (2) intensity cycling exercise. The vertical dotted line denotes the onset of exercise from a 10 W baseline. Data are mean and SD and 30-s averages. * denotes $P < 0.05$, i.e. significant mean difference between CF patients and healthy controls, whilst + denotes a statistical trend ($p = 0.07$).

